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Arbutus unedo L. fruits stabilization during conservation in a sweet environment

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Arbutus unedo L. is an important food on the nutritional side. Unfortunately, most of its production is lost by the lack of appropriate conservation techniques. This study was carried out to follow the evolution of physicochemical, microbiological and microscopic parameters of two stages of Arbutus unedo L. fruits maturation during a period of conservation between 01 February 2021 and 29 July 2021 in a sugar solution with the presence of a percentage of lemon juice 4%. The results revealed that the fruits soaked in a sugar solution with a concentration of 75% remained more stable during the 180 days of conservation than those soaked in an equal concentration solution, 50% of sugar. With a slight increase in pH and medium pH, accompanied by a significant decrease in water content and an increase in total aerobic mesophilic germs. Therefore, sugar improves the conservation of Arbutus unedo L. fruits in a high concentration.
Keywords: *Arbutus unedo* L., conservation, soaking, sweet medium, ripening stage, physicochemical parameter.

INTRODUCTION

Arbutus unedo L. or strawberry tree, and locally known in Morocco as "sasnou". It is a fruit plant belonging to the Ericaceae family (Zitouni *et al.,* 2020), native to the Mediterranean region, mainly in North Africa, Western Europe, the Canary Islands and Western Asia (Lopes *et al.,* 2012; Kim, 2012). It can be found in an altitudes ranging from 600 to 1200 m, where the cold is not very usual and the summer drought is not very intense (Celikel *et al.,* 2008).
The fruit of Arbutus unedo is a pretty red spherical berry. Vary considerably in size, from 1 to 2 cm in diameter (Ayaz *et al.,* 2000), and an average mass of 3 to 5g (Tonelli and Gallouin., 2013). Mature arbutus fruits contain in their composition a large amount of sugar ranging from 42 to 52% of dry weight (Sagbas *et al.,* 2020; Alarcão-E-Leitão *et al.,* 2001); as well as several important antioxidant components, such as anthocyanins, ellagic acid derivatives, flavonols and proanthocyanidins (Pallauf *et al.,* 2008). In addition, vitamins such as vitamin C, E, carotenoids, β-carotene and β-crypto xanthine (Pawlowska *et al.,* 2006).
But despite this nutritional richness and antioxidant importance, the Arbutus unedo. L fruits remain neglected and have not yet had the place it deserves in scientific research; they are well known only in traditional and folk medicine (Mariotto *et al.,* 2008).
This neglect affects their distribution in the market and sometimes remains unknown in many cities in Morocco. Indeed, the consumption of fresh fruit is rare, being commonly used in the production of alcoholic drinks, jam or marmalade (Simonetti *et al.,* 2008).
In this sense, the great problem of all food companies is to find a method of preservation that keeps the taste, nutritional properties, texture and color characteristics and meets the health and commercial standards simultaneously.
In order to increase the shelf life of Arbutus unedo. Soaking in a sweet solution remains a simple and practical preservation method. Sugar is mainly formed by a component called sucrose (Frank Baker *et al.,*2012). Sucrose is an organic molecule composed of carbon, hydrogen and oxygen. It can be found in sugar cane as well as sugar beet. With a taste enhancer property, sugar is a preservative (Rux *et al.,* 2017). Indeed, its hygroscopic power allows it to fix water around it; once fixed, water is no longer free, so the AW value decreases. Thus, the microorganisms do not have enough free water to develop (Dias *et al.,* 2017).
Our work will be focused on the study of physicochemical, microbiological and microscopic parameters of two stages of ripening of Arbutus unedo fruits preserved for 180 days in a sugar solution, with two different concentrations of 50 and 75% of sugar, with a percentage of citric acid as an additive.

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MATERIALS AND METHODS

***Plant material*:** The fruits of Arbutus unedo L. used for all the experiments of this research were harvested on 30 January 2021, near EL Ksiba, more precisely (Tadaout forest, Taghbalout Nouhlima), which belongs to the region of Beni Mellal Khenifra with an altitude of 1338 m, and latitude and longitude respectively 32°31'N and 6°11'W. In addition, the latter is characterized by a sub-humid climate with an average annual temperature reaching up to 16.4°C and precipitation equal to 718 mm per year (Rahima *et al.*, 2019).
Table 1 groups together the geographical and ecological characteristics of the EL ksiba zone. The fruits studied were purchased on the same day of their harvest. After two days of conservation in a refrigerator (-2 °C) the fruits of arbutus unedo were carefully transported to the laboratory to prepare their conservation conditions.

Table 1. The geographical and ecological characteristics of the harvesting zone of the samples

Harvest zone	EL ksiba
Region	Beni Mellal Khenifra
Province	Beni Mellal
Geographic region	Middle Atlas
Forest name	Tadaout forest, Taghbalout Nouhlima
Latitude	32°31'N
Longitude	6°11'W
Altitude	1338 m
Average annual temperature	16.4°C
Average annual precipitation	718 mm
Bioclimatic	sub-humid

***Experimental Approach*:** In order to study the stability of Arbutus unedo L. fruits during storage, the present study aims at soaking the studied fruits in a sugar juice in the presence of a percentage of citric acid, with a change of two essential factors: the concentration of sugar and the stage of maturity of the fruits.
Before storage, the fruits underwent several treatments: washing, selection according to their intermediate and mature ripe state, and physicochemical, microbiological and microscopic analyses. In addition, the preparation of two syrups with different concentrations (50 and 75% sugar) with water as solvent, and the addition of 4% of lemon juice (Ezoua *et al.*, 2008). The mixture will be poured into sterilized glass tanks with a volume of 175 ml.
Finally, soak 30 fruits in each tank and keep them in the ambient conditions of the laboratory, conditioning in which the tanks put them in the scrambling water for 1min. Every 60 days, a number of the fruits are removed and submitted to the analysis of each of the physicochemical, microbiological and microscopic parameters described below. Fig.1 summarizes the stages of fruit preservation.

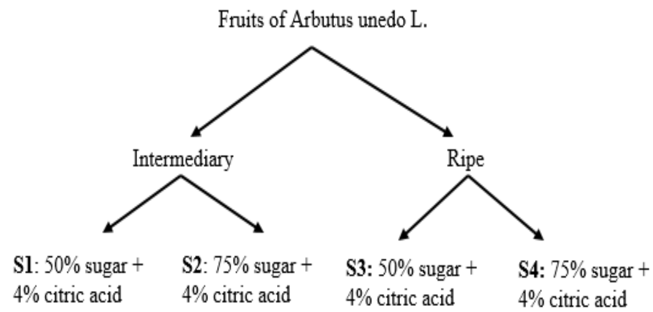


Figure 1. Manufacturing diagram of the different fruit preservation medium

Physicochemical Analysis: pH And Middle pH The pH of the medium was determined by direct measurement by dipping the electrode in the solution using a pH meter. To determine the pH of the fruits, 10g of the fruit cut into small pieces is weighed, then 100 ml of distilled water is added until juice is obtained. Furthermore, the measurement is made with a pH meter (AOAC, 2000).
***Titratable acidity*:** It consists of titrating the acidity of an aqueous solution with a 0.1 N sodium hydroxide solution in the presence of phenolphthalein as a colored indicator. The latter concerns the malic acid content (AOAC, 2002).
***Moisture content*:** The moisture content can be explained by weight loss during drying, using a drying of 2g of plant material in an oven at 103±2 °C and atmospheric pressure until a constant mass of the sample is obtained (Helrich, 1990).
***The total mass of fruits*:** The mass of the fruits was evaluated every 60 days and measured using an accurate balance (RADWAG, AS220.R2) with an error of 0.001g (Brewer *et al.*, 2006).
***Dry matter*:** The dry matter is determined by the same working condition in determining the moisture content, using a drying of 2g of plant material in an oven at 103±2 °C and atmospheric pressure (NF V05-105) (Chniti *et al.*, 2014).
***Study of bacterial activity*:** To enumerate total aerobic mesophilic germs, a stock solution contains 1g of plant material with 10 ml of distilled water; then, 1 ml of stock solution is taken to prepare other decimal dilutions. Then, 0.5ml of each dilution is poured into sterilized petri dishes containing a solid surface of 15ml of agar (PCA) and incubated in an oven set at 37 °C for 72 ± 3 hours. Finally, the enumeration of the plates containing between 15 and 300 colonies (Norme NF.V08.011., 1998 and Norme NF.V08.051.)
***Microscopic examination*:** In a blade, we deposit a very finely cut fragment of fruit, then we add a drop of distilled water, and finally, put a lamella (Monteuuis, 1989).

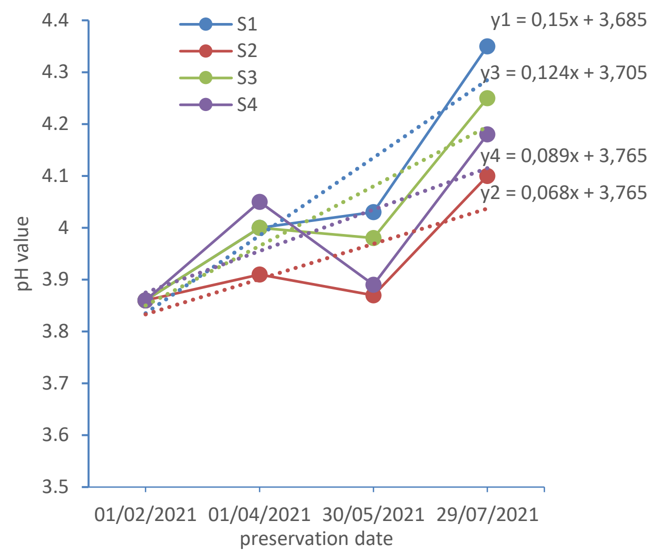
Observing the montage with a microscope (OPTIKA Microscope) at ×10 and then ×40 magnification and taking images for each magnification.

RESULTS AND DISCUSSION

The results of the physicochemical and microbiological parameters of Arbutus unedo. L fruit of the EL ksiba zone (Maroc) during the 180 days of conservation in sugar juice. Table 2 summarizes the results obtained.

***pH and Middle pH*:** The evolution of the pH value of fruits during preservation is mainly related to the strength of the acids present in Arbutus unedo L. fruits and to the value of the medium pH (Musa Özcan and al. 2007), in which a percentage of lemon juice is used as an additive.

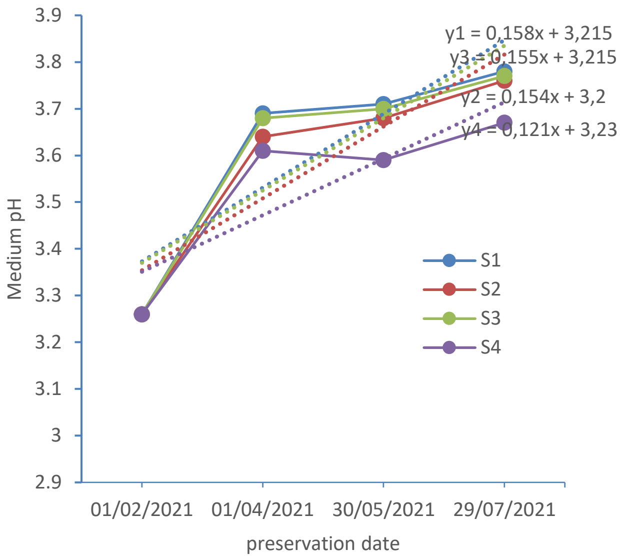
The results of this experiment are recorded in Graphs 1 and 2. According to the results obtained in graph (1), it is noticeable that at the beginning of the experiment, the pH values of the fruits are very close to 3.86 for those of the intermediate stage and 3.83 for the fruits of mature arbutus unedo. Then they increase in the first 60 days of conservation with an average value of 0.14, 0.05, 0.17 and 0.22 for samples S1, S2, S3 and S4, respectively. Subsequently, a plateau is obtained in the second 60 days of conservation and the pH value remains relatively constant for the different samples; this indicates that



Graph 1. pH variation of Arbutus unedo L. fruit during storage. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid); y1, y2, y3 and y4 represent the evolution equations of S1, S2, S3 and S4 respectively.

Table 2. The evolution of the physicochemical parameters of the fruits of Arbutus unedo L. during conservation. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid).

Parameters	Samples	01/02/2021	01/04/2021	30/05/2021	29/07/2021
pH value	S1	3.86±0.01	4.00±0.020	4.03±0.010	4.35±0.010
	S2	3.86±0.01	3.91±0.010	3.87±0.010	4.10±0.015
	S3	3.83±0.01	4.00±0.010	3.98±0.005	4.25±0.010
	S4	3.83±0.01	4.05±0.020	3.89±0.005	4.18±0.015
Medium pH	S1	3.26±0.01	3.69±0.010	3.71±0.015	3.78±0.010
	S2	3.26±0.01	3.64±0.015	3.68±0.015	3.76±0.015
	S3	3.26±0.01	3.68±0.015	3.70±0.010	3.77±0.015
	S4	3.26±0.01	3.61±0.015	3.59±0.010	3.67±0.020
Titratable acidity (%)	S1	0.60±0.06	0.50±0.010	0.49±0.010	0.42±0.020
	S2	0.60±0.06	0.52±0.015	0.55±0.010	0.51±0.010
	S3	0.62±0.06	0.51±0.015	0.52±0.015	0.45±0.015
	S4	0.62±0.06	0.48±0.020	0.55±0.025	0.46±0.250
Moisture content (%)	S1	60.75±2.33	67.33±2.510	64.50±0.700	66.70±0.350
	S2	60.75±2.33	47.00±1.730	48.00±0.010	50.75±1.060
	S3	63.66±3.21	56.33±1.520	61.50±0.700	67.00±1.410
	S4	63.66±3.21	44.33±1.020	43.50±2.120	45.00±0.100
Total mass (g)	S1	4.69±1.38	4.27±0.760	4.70±0.600	4.75±1.900
	S2	4.88±2.01	3.38±1.250	3.29±0.960	3.41±0.930
	S3	4.55±1.76	4.17±2.060	4.19±0.600	4.61±0.740
	S4	4.78±1.25	3.24±0.670	3.21±0.650	4.25±0.670
Dry matter	S1	39.25±2.27	32.67±2.310	35.50±1.020	33.30±0.670
	S2	39.25±2.27	53.00±1.750	52.00±0.060	49.25±1.700
	S3	36.34±3.24	43.67±1.620	38.50±0.500	33.00±1.540



Graph 2. pH medium variation of Arbutus unedo L. fruit during storage. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid); y1, y2, y3 and y4 represent the evolution equations of S1, S2, S3 and S4 respectively

we have a stabilization in all the conservation media. However, from 120 days, the pH of the fruits begins to increase until reaching a maximum value at the end of conservation; taking into account that this increase reaches 0.32, 0.14, 0.27 and 0.29 for the fruits of tanks S1, S2, S3 and S4 respectively.

If we compare these results with the average values of the increase of pH from the beginning to the end of the conservation 0.49, 0.24, 0.42 and 0.35 for S1, S2, S3 and S4, respectively, we notice that the increase of pH at the last 60 days represents about 50% for the global values.

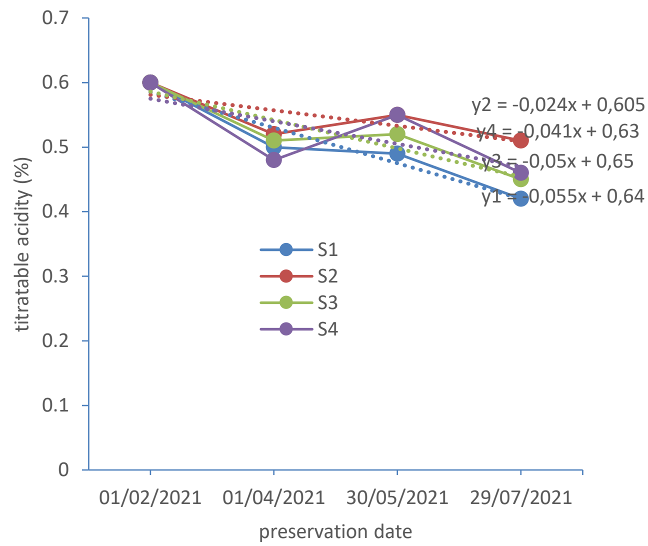
In general, whatever the concentration or the stage of ripening of the fruits, the pH value increases over time; but this increase remains less important for the two samples, S2 and S4, compared to S1 and S3. The sugar concentration significantly affects the evolution of pH and graph (1) represents the equation of the linearity of the latter.

Returning to the results mentioned in graph (2), which shows a remarkable increase in the pH of the medium at the first 60 days of conservation, reaching 0.43, 0.38, 0.42 and 0.35 for samples S1, S2, S3 and S4, respectively. While at the time of 60 to the end of conservation, we will have a slight increase for the four samples. There would thus be a parallel evolution between the pH of the fruits and the pH of the media.

It can be concluded that despite this increase in fruit pH level, our results are still lower than that obtained by Şeker *et al.* (2010) and Serçe *et al.* (2010) which are 5±0.3 and 5.57±0.07 respectively for the pH of fresh Arbutus unedo fruits.

***Titratable Acidity*:** The acidity of food products plays a more important role in the growth of microorganisms and sensory properties (Siebert *et al.* 1999). It provides information on the quantity of organic acids present in the sample.

The following graph (3) shows the evolution of this parameter during conservation.



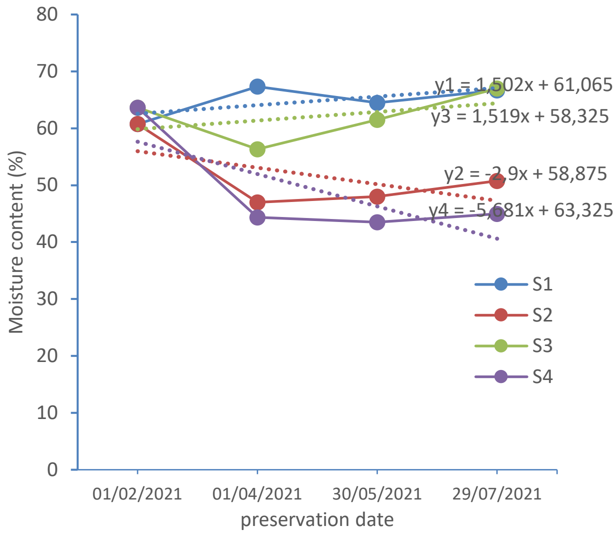
Graph 3. Titratable acidity variation of Arbutus unedo L. fruit during storage. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid); y1, y2, y3 and y4 represent the evolution equations of S1, S2, S3 and S4 respectively

It can be seen from the curves in graph (3) that the acidity of the fruit decreases during storage differently for the four samples. This decrease is remarkable, especially in the first 60 days of conservation in which it reaches 0.18, 0.09, 0.17 and 0.16 for samples S1, S2, S3 and S4 respectively. And continues to decrease, arriving at values of 0.42, 0.51, 0.45 and 0.46 for S1, S2, S3 and S4 respectively. In addition, graph (1) results show that the titratable acidity of the fruits of arbutus unedo varies in the opposite direction than the pH.

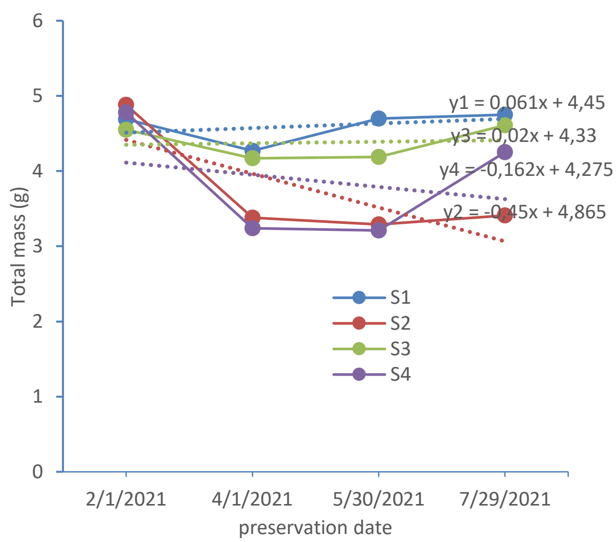
The titrated values of the four preserved samples are higher than those found by Musa Özcan *et al.*, (2007) which are 0.4±0.1%, and lower than 0.67±0.17% found by Serçe *et al.*, (2010).

***Moisture Content And Total Mass*:** Water is one of the essential constituents of the fruit. It has a fundamental importance on the quality of food and acts on its preservation. In addition, the relationship between water activity and the total mass of fruits is studied.

The following graphs (4) (5) represent their evolution during the 180 conservation days.



Graph 4. Moisture content variation of Arbutus unedo L. fruits during storage. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid); y1, y2, y3 and y4 represent the evolution equations of S1, S2, S3 and S4 respectively



Graph 5. *T*otal mass variation of Arbutus unedo L. fruit during storage. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid); y1, y2, y3 and y4 represent the evolution equations of S1, S2, S3 and S4 respectively

According to the results of the pH, medium pH and titratable acidity, we notice a huge change in the first 60 days of the conservation graph (4), in which we have noticed a significant decrease for the samples S2 and S4 of 13.75 and 19.33% respectively, and remains stable in the last 120 days of conservation, until reaching an average decrease of 10 and 18.66 g, which implies that there is a slight increase of the moisture content in the last days of conservation. On the other hand, a less significant increase is underlined, from 60.75 to 66.7 and from 63.66 to 67, respectively for the samples S1 and S2.

The same is true for the results of the evolution of the total mass of Arbutus unedo fruits during storage graph (5). During the first 60 days of storage, the total fruit mass decreased by an average of 1.5 and 1.54 g for S2 and S4 respectively, and remained stable after 60 days until the end of storage. Against this decrease, we see a slight increase from 4.69 to 4.75 and 4.55 to 4.61 g for samples S1 and S3 respectively. This increase is due to the penetration of water in the fruit of samples S1 and S3, contrary to the fact that we can say that we have an escape of water from the fruit to the sweet medium. And these results are compatible with the moisture content, in which we notice that the penetration of water affects on the increase of the mass and automatically on the moisture content and the opposite.

In general, and according to graphs 5 and 6, we can see that the evolution of the moisture content and the total mass of the fruits is similar for samples S1, S3 and S2, S4, which implies that the sugar concentration significantly influences both parameters.

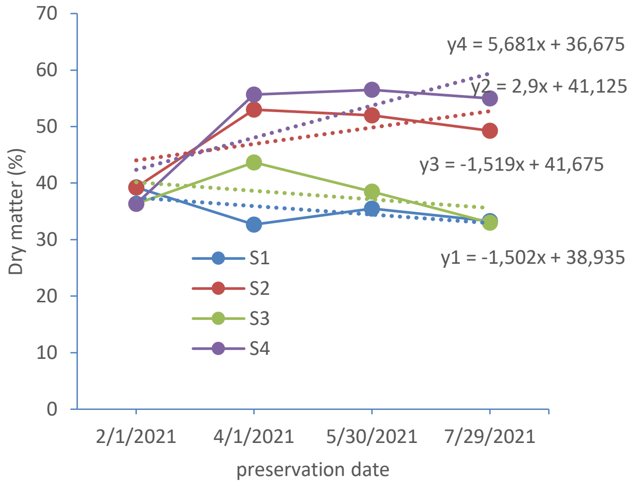
To conclude, the high concentration causes a decrease in moisture content with a displacement of free water responsible for developing microorganisms from the fruit to the external environment.

***Dry matter*:** The dry matter is a very important parameter in the food, in which it gives an idea about the quantity of water lost and at the same time about the nutritional value.

The following graph (6) represents the evolution of the dry matter in function of the time.

According to the results presented in graph (6), it can be seen that the percentage of dry matter increases rapidly in the first 60 days of conservation, followed by a slight decrease in dry matter to 49.25±1.7 and 55±0,75 on the last day of conservation, for samples S2 and S4 respectively. On the other hand, a decrease in dry matter is observed over time ranging from 39.25±2.27 to 33.3±0.67 and from 36.34±3.24 to 33±1.54 for samples S1 and S3 respectively.

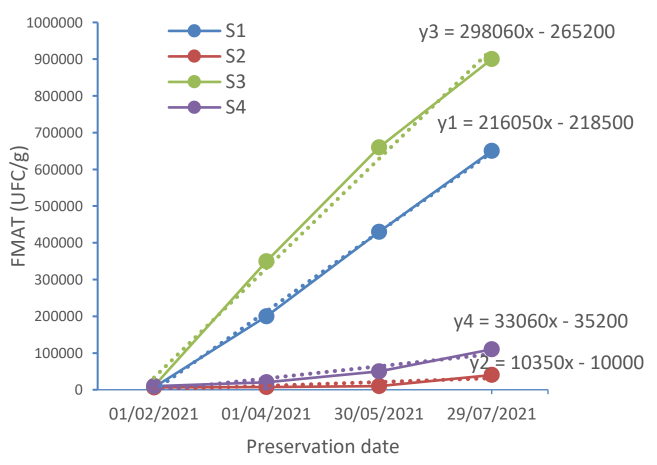
These results show that the dry matter of the fruits soaked in a solution containing a percentage of sugar to 75% increases due to the displacement of water to the external environment, and contrary to the solution of 50% in sugar. While these proposals are superposable with the results mentioned in graphs 4 and 5.



Graph 6. Dry matter variation of Arbutus unedo L. fruit during storage. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid); y1, y2, y3 and y4 represent the evolution equations of S1, S2, S3 and S4 respectively

***Microbiological activity study*:** Microbiological criteria are mandatory in the food sector. Respecting the acceptability of a food in terms of health that apply to products placed on the market.

The graph below (7) represents the evolution of total aerobic mesophilic flora while conserving Arbutus unedo fruits.



Graph 7. FMAT evolution of Arbutus unedo L. during storge. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid); y1, y2, y3 and y4 represent the evolution equations of S1, S2, S3 and S4 respectively

The dismemberment of microorganisms present in arbutus fruits during the 180 days of preservation is reported in Table 1 of graph (7). In there is a slight increase for samples S2 and S4 vary from 65×10^2 to 4×10^4 and from 98×10^2 to 11×10^4 UFC/g respectively. There is also a gradual increase in this criterion from the first 60 days to the end of storage ranging from 65×10^2 to 65×10^4 and from 98×10^2 to 90×10^4 UFC/g for samples S1 and S3 respectively.

In addition, a similar evolution is clearly observed for the samples soaked in the same sugar concentration S2, S4 and S1, S3. However, during storage, a low sugar concentration has a positive influence on the development of the bacteria; and the speed of development of the latter are represented in graph (8) under a linear curve.

Despite this significant increase and whatever the sugar concentration or the degree of maturity of the fruits, this food product remains comfortable based on the Quebec 2009 standard, which requires a value of 10^7 CFU/g for total aerobic mesophilic germs (Norme Québec, 2009).

***Microscopic examination*:** The image analysis procedure was based on plant cells and their evolutions during conservation. Photos take through a microscope at the beginning of the experience (S1, S2, S3 and S4) and after 180 days of conservation (S1', S2', S3' and S4').

By analyzing the above figure (2), it is clear that the red coloration of the cells which gives color to the arbutus fruits disappeared totally after 180 days of conservation for the sample S1, S2, S3 and S4. Thus, the Arbutus unedo fruits lose their color during storage regardless of the sugar concentration and the ripening stage, which may negatively influence the acceptability of the fruits to the customers (Armenteros *et al*., 2013).

From the shape of the plant cells, we can see that the fruits preserved in a liquid medium with a concentration of 75% of sugar are undergoing plasmolysis (S2', S4'), due to the passage of free water from the cell to the external medium; this means the passage from a less concentrated medium to a more concentrated one. In contrast, cells kept in a sugar medium with a concentration of 50% are turgid (S1', S3'), due to the penetration of water from the less concentrated external medium into the cells.

Following the results of graphs 4 and 5, the decrease in moisture content and total mass for samples S2 and S4 is due to the displacement of free water to the external medium, as shown in the photos of the plant cells for samples S2' and S4' figure (2). On the other hand, the turgid cells indicated in the photos S1' and S3' figure (2), we can explain this phenomenon by the penetration of water in the cells of the fruits; based on the results of graphs 4 and 5 that show the increase of the water content and the total mass.

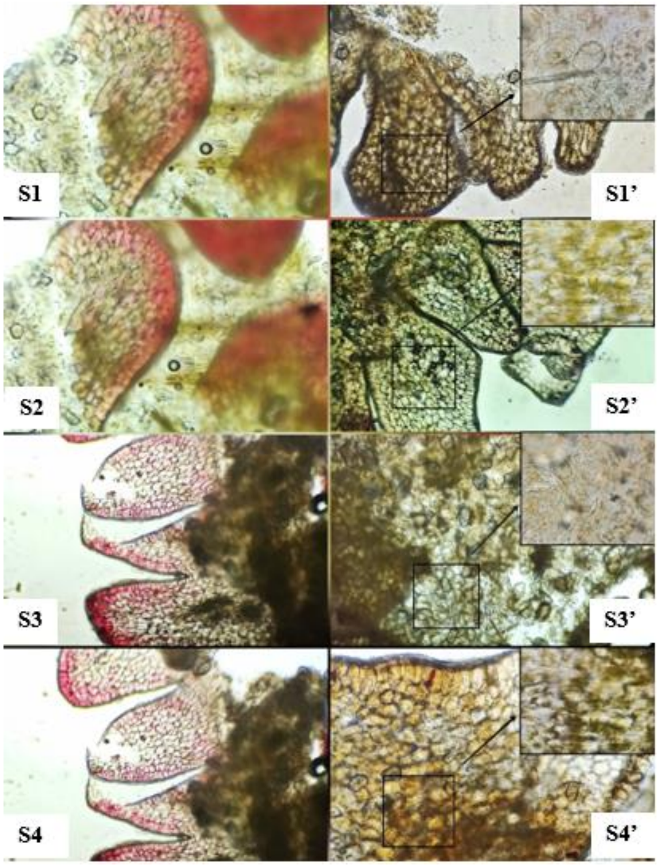


Figure 2. Mmicroscopic photos of plant cells observed before and after 180 days of storage. Intermediary (S1: 50% sugar+4% citric acid; S2: 75% sugar+4% citric acid); Ripe (S3: 50% sugar+4% citric acid; S4: 75% sugar+4% citric acid). S1, S2, S3 and S4 before storage, after 180 days of storage (S1', S2', S3' and S4'

***Conclusion*:** Arbutus unedo is native to the Mediterranean countries, it remains seasonal and less widespread in the markets and almost non-existent in several cities of Morocco; because of their sensitivity. Monitoring the evolution of physicochemical, microbiological and microscopic parameters during the preservation of the strawberry fruits allowed to highlight the efficiency of the studied factors (sugar concentration and the degree of maturity of the fruits). In general, whatever the fruit ripening stage, however, the best results are obtained with a sugar concentration equal to 75%. In obtains a slight increase of pH arrives until 4.1 and 4.18 for the intermediate and mature stage, followed by a decrease of titratable acidity, moisture content and total mass. Finally, the total aerobic mesophilic germs level increases but remains comfortable to the norm. This simple and practical preservation method allows for increasing the shelf life of this fruit without chemical additives infecting the health.

Authors' Contributions statement: B. El Mejhed; Writing – Original draft preparation – Data curation- Formal Analysis – Funding Acquisition, F. Kzaibre; Review- Supervision, W. Terouzi; Review – Supervision – Project Administration – Resources.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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